

# Association of Pathogen Burden and Hypertension: The Persian Gulf Healthy Heart Study

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## BACKGROUND

Chronic infection with cytomegalovirus (CMV), *Chlamydia pneumoniae*, herpes simplex virus 1 (HSV-1), and *Helicobacter pylori* may contribute to essential hypertension. However, the evidence now available does not clarify whether the aggregate number of pathogens (pathogen burden) may be associated with hypertension.

## METHODS

Sera from 1,754 men and women aged  $\geq 25$  years were analyzed for immunoglobulin G antibodies to *C. pneumoniae*, HSV-1, *H. pylori*, and CMV using enzyme-linked immunosorbent assay. The aggregate number of seropositives to the studied viral and bacterial agents was defined as pathogen burden. Hypertension was defined according to World Health Organization criteria.

## RESULTS

A total of 459 (26.3%) of the subjects had hypertension. In the hypertensive group, 4.2% had 0 or 1 pathogens present, 20.6% had 2, 43.2% had 3, and 32.1% had 4; in the normotensive group, 7.9% had 0 or 1, 28.4% had 2, 42.7% had 3, and 21.0% had 4. Of the 4 studied pathogens,

*H. pylori* seropositivity showed a significant independent association with hypertension (odds ratio (OR) = 1.37; 95% confidence interval (CI) = 1.05–1.79;  $P = 0.02$ ). In multiple logistic regression analyses, the pathogen burden did not show a significant independent association with hypertension. Coinfection with *H. pylori* and *C. pneumoniae* was significantly associated with hypertension compared with double seronegativity after adjustment for age, sex, chronic low-grade inflammation, and cardiovascular risk factors (OR = 1.68; 95% CI = 1.14–2.47;  $P = 0.008$ ).

## CONCLUSIONS

The pathogen burden was not associated with hypertension. However, coinfection with *C. pneumoniae* and *H. pylori* showed a significant association with essential hypertension, independent of cardiovascular risk factors and chronic low-grade inflammation.

**Keywords:** blood pressure; *Chlamydia pneumoniae*; cytomegalovirus; *Helicobacter pylori*; herpes simplex virus; hypertension; pathogen.

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Chronic infection with *Chlamydia pneumoniae*, *Helicobacter pylori*, and persistent viruses such as herpes simplex virus 1 (HSV-1) and cytomegalovirus (CMV) has gained considerable interest as a contributing factor in atherosclerosis.<sup>1–3</sup>

More recently, the results of some seroepidemiological and prospective cohort studies showed that the aggregate number of pathogens (infectious burden), rather than any single organism, may contribute to coronary artery disease<sup>1,4</sup> and stroke.<sup>5</sup> This concept has been referred to as pathogen burden (infectious burden).<sup>6</sup>

The evidence now available does not clarify whether pathogen burden per se may be involved in atherosclerosis

either through induction of an inflammatory and procoagulant milieu in the vessels or through the indirect effects of cytokines or acute phase proteins, promoted by pathogens at “nonvessel” sites.<sup>2,3,6</sup>

Interestingly, beyond the known traditional risk factors for hypertension, chronic infection with CMV<sup>7,8</sup>, *C. pneumoniae*,<sup>9–11</sup> HSV,<sup>12</sup> and *H. pylori*<sup>13,14</sup> has recently been reported in association with high blood pressure, suggesting that pathogens may contribute to essential hypertension.

The synergistic effects of multiple infectious agents in atherosclerosis poses new questions about the link between the aggregate numbers of pathogens to which an individual

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has been exposed and hypertension. In parallel to work linking pathogen burden to cardiovascular disease, Liu *et al.*<sup>15</sup> showed that essential hypertension was associated with the aggregate pathogens in a cross-sectional study. However, the results of that study have not yet been evaluated in further investigations.

Therefore, we investigated the association of pathogen burden and hypertension, regarding other cardiovascular risk factors, in a large-scale, community-based study in an Iranian population.

## METHODS

### Community sampling and baseline examinations

We conducted this study as part of the Persian Gulf Healthy Heart Study, which was a prospective, population-based, cohort study started in 2003–2004 using men and women aged >25 years. Detailed information about the methods and procedures of this study is available elsewhere.<sup>16</sup>

In a study ancillary to the Persian Gulf Healthy Heart Study, a total of 1,754 subjects (49.2% male, 50.8% female) were selected through a stratified multistage design process from major ports of Bushehr Province (the Iranian province having the greatest border with the Persian Gulf). All subjects were asked to fast and to present to the survey center between 7:30 AM and 9:30 AM. After a 15-minute rest in the sitting position, blood pressure was assessed twice at the right arm using a standard mercury sphygmomanometer. Height and weight were measured using a stadiometer. Heavy outer garments and shoes were removed before measuring height and weight. Body mass index (BMI) was calculated.

### Biochemical and serologic measurements

A fasting blood sample was taken, and all samples were promptly centrifuged, separated, and analyzed at the Persian Gulf Health Research Center on the day of blood collection using a Selectra 2 autoanalyzer (Vital Scientific, Spankeren, the Netherlands).

Glucose was assayed by the enzymatic (glucose oxidase) colorimetric method using a commercial kit (Pars Azmun, Tehran, Iran). Serum total cholesterol and high-density lipoprotein cholesterol were measured using a cholesterol oxidase phenol aminoantipyrine, and triglycerides were measured using a glycerol-3 phosphate oxidase phenol aminoantipyrine enzymatic method. Serum low-density lipoprotein (LDL) cholesterol was calculated using the Friedwald formula; LDL cholesterol was not calculated when triglycerides concentration was >400 mg/dl. Measurement of CRP was performed by a high-sensitivity C-reactive protein (hs-CRP) assay, CRP HS ELISA (DRG International, Springfield, New Jersey, USA).

Immunoglobulin G (IgG) antibodies against *C. pneumoniae* were measured by a commercial test kit (DRG Instruments, Marburg, Germany). The principle of the kit was based on an indirect solid-phase enzyme immunoassay with horseradish peroxidase as a marker enzyme; the positivity threshold was enzyme immuno-units >45. Sera were screened for IgG antibodies against HSV, CMV, and *H. pylori* with an enzyme-linked immunosorbent assay kit (Radim

SpA, Pomezia RM, Italy), and the samples were considered positive with IgG values >30 RU/ml for CMV and *H. pylori*. Samples with optical density higher than the cutoff control were considered reactive for anti-HSV type 1 IgG antibodies.

## Definitions

Hypertension was defined according to World Health Organization criteria (systolic blood pressure  $\geq 140$  mm Hg, diastolic blood pressure  $\geq 90$  mm Hg, or use of antihypertensive medication).<sup>17</sup> The mean arterial pressure was estimated using conventional formula: mean arterial pressure = (2 diastolic blood pressure + systolic blood pressure)/3.<sup>18</sup> Using American Diabetes Association criteria, a fasting plasma glucose of  $\geq 126$  mg/dl or the use of antidiabetic measures was defined as diabetes.<sup>19</sup> The cutoff points of serum total cholesterol, high-density lipoprotein cholesterol, and LDL cholesterol distributions used to assign subjects to different levels of risk were derived from the National Cholesterol Education Program guidelines in the United States (Adult Treatment Panel III).<sup>20</sup> Smoking was considered to be present when the participant smoked cigarettes or used a shisha (water pipe) daily.

The aggregate number of seropositives to *C. pneumoniae*, *H. pylori*, cytomegalovirus, or HSV-1 was defined as pathogen burden.<sup>6,21</sup>

## Statistical methods

Normal distribution of the data was controlled with the Kolmogorov–Smirnov test. The significance of the difference in results of any 2 groups was determined by  $\chi^2$  analysis using 2  $\times$  2 contingency tables for categorical variables. A 2-tailed *t* test was used to compare mean values across groups. We found that log transformation of hs-CRP gave a better fit to a Gaussian distribution. The geometric mean for hs-CRP was defined as the arithmetic mean of the log-transformed data  $\pm 2$  standard deviations, raised to the power of 10.

Binary logistic regression models were used to assess association between IgG seropositivities to common bacterial and viral agents, pathogen burden (4, 3, or 2 pathogens vs. 0 or 1 pathogen) or coinfections and hypertension (as a dependent variable). Further, the models were adjusted for BMI, smoking, log-transformed CRP levels, type 2 diabetes mellitus, high triglycerides, low high-density lipoprotein cholesterol, and high LDL cholesterol in addition to age and sex. Known risk factors for cardiovascular diseases and characteristics related to hypertension were considered to be potential confounders and examined for inclusion in multivariable models.

The models' fits were measured with the Hosmer–Lemeshow statistic. The overall predictive ability of the models was assessed by use of the area under the receiver operating characteristic curve.

There were no significant interactions between seropositivities to pathogens and cardiovascular risk factors (e.g., obesity) with hypertension. Therefore, we didn't include these interaction terms in our final models.

Multiple linear regression models were used to assess the association between pathogen burden (independent

variables) and the estimated mean arterial pressure (dependent variable). Probability values  $<5\%$  were considered statistically significant. All statistical analyses were performed using the PASW Statistics GradPack 18 (SPSS, Chicago, IL).

## RESULTS

### Baseline characteristics

A total of 1,754 subjects (49.2% men, 50.8% women) aged 25–66 years were evaluated for associations of pathogen burden and hypertension. Mean age of participants was 40.79 (SD = 11.15) years.

Relevant anthropometric information including cardiovascular risk factors, biochemical measurements, and the prevalence of IgG antibodies against CMV, HSV, *C. pneumoniae*, and *H. pylori* are presented in Table 1.

Men had higher systolic ( $117.3 \pm 10.7$  vs.  $113.22 \pm 11.1$  mm Hg) and diastolic blood pressure ( $74.3 \pm 8.2$  vs.  $73.1 \pm 7.7$  mm Hg) than women ( $P < 0.0001$ ). A total of 459 (26.3%) subjects (30.8% of men and 21.8% of women;  $P < 0.0001$ ) had hypertension.

The prevalence of IgG antibodies against CMV ( $P = 0.004$ ), *C. pneumoniae* ( $P < 0.0001$ ), and *H. pylori* ( $P < 0.0001$ ) was higher in the hypertensive group than in healthy persons. However, there was no statistically significant difference in the prevalence of IgG antibodies against HSV between normotensive and hypertensive individuals ( $P = 0.29$ ) (Table 1). Women also had significantly higher seropositivity for HSV ( $P = 0.004$ ) but lower seropositivity for *C. pneumoniae* ( $P < 0.0001$ ) than men. However, there were no statistically significant differences in the prevalence of IgG antibodies against CMV ( $P = 0.07$ ) and *H. pylori* ( $P = 0.20$ ) between men and women.

In the hypertensive group, 4.2% had 0 or 1 pathogens present, 20.6% had 2, 43.2% had 3, and 32.1% had 4; in the normotensive group, 7.9% had 0 or 1, 28.4% had 2, 42.7% had 3, and 21.0% had 4. There was a marginally significant difference in the prevalence of number of pathogens between men and women ( $P = 0.04$ ).

### Association between single pathogen and hypertension

The age- and sex-adjusted odds ratio (ORs) between IgG seropositivity for *C. pneumoniae*, *H. pylori*, or CMV and hypertension was significant ( $P < 0.05$ ); however, only

**Table 1.** Clinical characteristics and laboratory values of a random population of the northern Persian Gulf (the study population) according to blood pressure status

	Normotensive (n = 1,295)		Hypertensive (n = 459)		P value <sup>a</sup>
	Men	Women	Men	Women	
Age groups, years, %					
25–34	43.8	46.1	18.5	14.4	$<0.0001$
35–44	33.5	30.5	24.9	21.1	
45–54	15.8	16.7	27.9	38.1	
55–66	6.9	6.6	27.9	25.3	
Body mass index, kg/m <sup>2</sup>	25.2 (4.1)	27.7 (5.4)	27.8 (5.2)	30.7 (6.0)	$<0.0001$
Total cholesterol, mg/dl	194.7 (44.4)	203.4 (46.5)	217.0 (47.3)	234.7 (48.9)	$<0.0001$
HDL cholesterol, mg/dl	41.1 (20.6)	48.8 (47.3)	42.0 (47.4)	45.3 (12.7)	0.17
LDL cholesterol, mg/dl	119.1 (42.4)	124.3 (60.3)	134.7 (61.7)	150.3 (43.2)	$<0.0001$
Triglycerides, mg/dl	172.1 (106.5)	150.93 (91.5)	201.3 (11.9)	195.2 (99.3)	$<0.0001$
Fasting blood sugar, mg/dl	87.7 (29.8)	89.4 (40.2)	99.7 (46.1)	106.6 (54.0)	$<0.0001$
Smoking, %	36.4	20.4	36.6	26.8	0.06
Physical inactivity, %	68.9	76.1	68.3	62.4	0.005
Obesity, %	9.4	30.9	29.4	53.6	$<0.0001$
High total cholesterol, %	16.0	20.1	27.9	40.2	$<0.0001$
Low HDL-cholesterol, %	56.9	38.1	60.0	37.6	0.17
High triglycerides, %	50.0	40.6	62.6	60.3	$<0.0001$
<i>Chlamydia pneumoniae</i> seropositive, %	42.4	34.0	54.0	42.3	$<0.0001$
<i>Helicobacter pylori</i> seropositive, %	60.2	57.9	69.4	68.0	$<0.0001$
Cytomegalovirus seropositive, %	90.6	93.5	95.5	96.9	0.004
Herpes simplex virus type 1 seropositive, %	83.1	87.7	84.8	91.5	0.29

Values are mean (SD), except for those indicated as being %. Hypertension was defined according to the World Health Organization. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

<sup>a</sup>P values indicate statistical significance of various clinical and biochemical indices between normotensive and hypertensive subjects.

**Table 2.** Prediction of hypertension by isolated pathogen seropositivity, pathogen burden, and coexisting infections using multivariable odds ratios and their 95% confidence intervals

	Adjusted for age and sex			Adjusted for full model <sup>a</sup>		
	OR	95% CI	P value	OR	95% CI	P value
<i>C. pneumoniae</i>	1.35	1.07–1.70	0.01	1.22	0.94–1.58	0.12
<i>H. pylori</i>	1.31	1.03–1.67	0.03	1.37	1.05–1.79	0.02
CMV	1.79	1.04–3.08	0.03	1.76	0.97–3.17	0.06
HSV-1	0.94	0.66–1.34	0.74	0.95	0.65–1.40	0.81
2 pathogens <sup>b</sup>	1.15	0.64–2.07	0.63	0.93	0.48–1.78	0.93
3 pathogens <sup>b</sup>	1.34	0.76–2.35	0.30	1.13	0.60–2.11	0.70
4 pathogens <sup>b</sup>	1.87	1.05–3.34	0.03	1.51	0.79–2.87	0.21
<i>H. pylori</i> + <i>C. pneumoniae</i>	1.73	1.23–2.42	0.002	1.68	1.14–2.47	0.008
<i>H. pylori</i> + CMV	1.31	0.91–1.89	0.14	1.28	0.86–1.90	0.22
<i>H. pylori</i> + HSV-1	1.01	0.76–1.31	0.97	0.97	0.72–1.31	0.89

The Hosmer–Lemeshow statistics were from 4.5 ( $P = 0.81$ ) to 10.96 ( $P = 0.20$ ), indicating good levels of fit for all models. The area under the receiver operating characteristic curves for the models were from 0.7847 to 0.8165.

Abbreviations: *C. pneumoniae*, *Chlamydia pneumoniae*; CMV, cytomegalovirus; *H. pylori*, *Helicobacter pylori*; HSV, herpes simplex virus type 1.

<sup>a</sup>Full model included age, sex, smoking, body mass index, and type 2 diabetes mellitus, high triglyceride, low high-density lipoprotein cholesterol, high low-density lipoprotein cholesterol, and log-transformed high-sensitivity C-reactive protein (hs-CRP) levels.

<sup>b</sup>Versus 0 or 1 pathogen.

*H. pylori* seropositivity retained its significant association with hypertension after adjusting for sex, age, dyslipidemia, smoking, type 2 diabetes mellitus, BMI, and hs-CRP (OR = 1.37; 95% confidence interval (CI) = 1.05–1.79;  $P = 0.02$ ) (Table 2).

#### Association between pathogen burden and hypertension

The association between age- and sex-adjusted infectious burden for 4 pathogens, compared with 0 or 1 pathogen, and hypertension was significant; however, this association was lost with further adjustments for cardiovascular risk factors (Table 2).

In multiple logistic regression analyses, the pathogen burden did not show a significant association with hypertension after adjusting for sex, age, dyslipidemia, smoking, type 2 diabetes mellitus, BMI, and hs-CRP (Table 2).

In linear regression analyses, age-adjusted pathogen burden did not show significant correlations with the estimated mean arterial pressure in both men ( $\beta = 0.05$ ;  $P = 0.12$ ) and women ( $\beta = 0.01$ ;  $P = 0.61$ ) (Figure 1).

#### Association between coinfection with *H. pylori* and *C. pneumoniae*, CMV, or HSV and hypertension

Age- and sex-adjusted coinfection with *H. pylori* and *C. pneumoniae* was significantly associated with hypertension compared with double seronegativity (OR = 1.73; 95% CI = 1.23–2.42;  $P = 0.002$ ). After adjustment for cardiovascular risk factors, including hs-CRP levels, the significant association of this coinfection remained (OR = 1.68; 95% CI = 1.14–2.47;  $P = 0.008$ ) (Table 2). However, *H. pylori* seropositivity in

combination with CMV or HSV seropositivity was not associated with hypertension in regression models (Table 2).

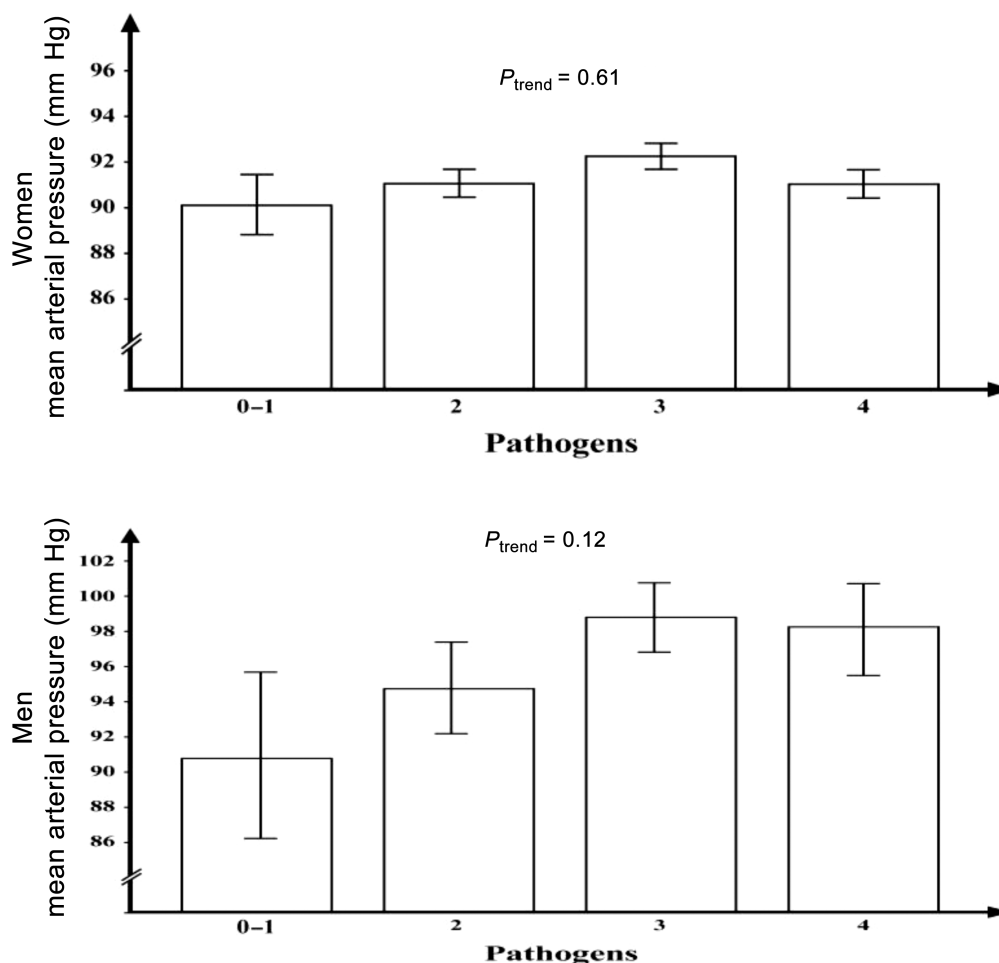
#### DISCUSSION

In this large, population-based study, there was no independent association between hypertension and the burden of common viral and bacterial pathogens that had been previously associated with coronary artery disease as well as carotid atherosclerosis. However, coinfection with *C. pneumoniae* and *H. pylori* was associated with high blood pressure, independent of cardiovascular risk factors and chronic low-grade inflammation.

The mechanisms by which infectious agents may contribute to atherosclerosis include infection-induced autoimmune response due to microbial molecular mimicry, enhanced uptake of cholesterol and of modified LDL cholesterol, augmentation of procoagulant and proinflammatory activities, endothelial dysfunction, alteration of apoptosis, and increased expression of adhesion molecules and scavenger receptors.<sup>1–3,6</sup>

According to a pathogen burden model, the greater the number of bacterial pathogens such as *H. pylori* and *C. pneumoniae* and exposures during one's lifetime to persistent viruses such as HSV and CMV, the higher the chance of atherosclerosis and its thrombotic complications that should be anticipated.<sup>1,22,23</sup> The impact of viral and bacterial infectious burden on long-term prognosis in patients with coronary artery disease<sup>1,4</sup> and stroke<sup>5</sup> has already been shown.

Evidence for the existence of a potential link between chronic low-grade inflammation and hypertension is growing.<sup>24</sup> Thus, it is plausible that pathogen burden would



**Figure 1.** Age-adjusted estimated mean arterial pressure (estimated marginal means  $\pm$  SE) across pathogen burden in men and women.

contribute to the risk of hypertension through infection-induced inflammatory response.

In contrast with the results of our study, in a cross-sectional study of 488 hypertensive and 942 normotensive Chinese Mongolian subjects, pathogen burden was associated with essential hypertension, whether adjusted by cardiovascular risk factors or not.<sup>15</sup>

Discrepancies between that study and our study might be due to differences in socioeconomic status, race/ethnicity, and the microorganisms studied. The study defined infectious burden as seropositives to *C. pneumoniae*, *M. pneumoniae*, *H. pylori*, and Coxsackie virus.<sup>15</sup> Therefore, the topic of pathogen burden in essential hypertension seems to be ill-defined and merits further studies.

The results of available studies for *C. pneumoniae*, *H. pylori*, HSV-1, and CMV in relation to hypertension are presented in Table 3.

In our study and the Liu *et al.* study,<sup>15</sup> the significant association between *C. pneumoniae* and hypertension was attenuated after further adjustment for cardiovascular risk factors. Conflicting data exist regarding *C. pneumoniae* in relation to hypertension. Some investigators reported a positive relationship between seropositivity to *C. pneumoniae* and hypertension.<sup>9,11</sup> However, others found no relationship,<sup>25,26</sup> or else

an inverse association of *C. pneumoniae* infection with high blood pressure.<sup>27</sup>

Herpes virus infections have been reported in association with human atherosclerosis in different types of studies.<sup>28,29</sup> In the Cardiovascular Health Study, seropositivity to HSV-1 was associated with a 2-fold increase in the risk of incidents of myocardial infarction and coronary heart disease death in older adults.<sup>29</sup>

There are several studies in the medical literature on the relationship of herpes virus infection to hypertension. In our study, we did not find a significant association between the presence of IgG antibodies to HSV-1 and hypertension. Kristensen *et al.*<sup>30</sup> reported an incremental trend toward HSV antibody positivity among subjects in different stages of hypertension. In a histopathological study,<sup>28</sup> herpes viral DNA was significantly associated with arterial hypertension. In a cross-sectional study, after adjustment for confounding factors, an association was found between HSV-2 infection and essential hypertension.<sup>12</sup> HSV-induced vascular inflammation and arterial wall thickening leading to the resistance of blood vessels were postulated as the underlying mechanisms.<sup>12</sup>

Likewise, little is known about CMV's role as a potential risk factor for hypertension.<sup>7,8,31</sup> In an *in vivo* experimental study, Cheng *et al.*<sup>31</sup> showed that CMV infection alone



**Table 3.** Available studies for *Chlamydia pneumoniae*, *Helicobacter pylori*, herpes simplex virus type 1 (HSV-1), and cytomegalovirus (CMV) in relation to hypertension

Pathogen	Investigators	Type of study	Number of participants	Relationship
<i>C. pneumoniae</i>				
	Liu <i>et al.</i> <sup>15</sup>	Cross-sectional survey	1,430	No
	Pitiriga <i>et al.</i> <sup>9</sup>	Case-control	170	Yes
	Blanc <i>et al.</i> <sup>10</sup>	Cross-sectional, population-based	1,304	Yes
	Cook <i>et al.</i> <sup>11</sup>	Case-control	246	Yes
	Coles <i>et al.</i> <sup>25</sup>	Cross-sectional, population-based	1,034	No
	Koh <i>et al.</i> <sup>26</sup>	Cross-sectional, population-based	1,068	No
	Nishimura <i>et al.</i> <sup>27</sup>	Case-control	229	Inverse
	Vahdat <i>et al.</i> (this study)	Cross-sectional, population-based	1,754	No
HSV-1				
	Kristensen <i>et al.</i> <sup>30</sup>	Case-control	186	Yes
	Sun <i>et al.</i> <sup>12</sup>	Case-control	1,244	Yes
	Ibrahim <i>et al.</i> <sup>28</sup>	Histopathological study	48	Yes
	Vahdat <i>et al.</i> (this study)	Cross-sectional, population-based	1,754	No
CMV				
	Cheng <i>et al.</i> <sup>31</sup>	<i>In vivo</i>		Yes
	Li <i>et al.</i> <sup>7</sup>	Cross-sectional, population-based	6,303	No in men Yes in women
	Haarala <i>et al.</i> <sup>8</sup>	Cross-sectional, population-based	1,931	Yes
	Vahdat <i>et al.</i> (this study)	Cross-sectional, population-based	1,754	Yes
<i>H. pylori</i>				
	Lip <i>et al.</i> <sup>13</sup>	Case-control	162	Yes
		Community-based, cross-sectional	4,900	No
	Vahdat <i>et al.</i> (this study)	Cross-sectional, population-based	1,754	Yes

caused a significant increase in arterial blood pressure. They also demonstrated that CMV infection activates overexpression of renin and angiotensin II in blood and in vessel cells in a persistent infection manner.<sup>31</sup>

In our study, we found a negligible, marginal association between the presence of IgG antibodies for CMV and hypertension, even when adjusting for atherosclerotic risk factors. But, this association was markedly attenuated by including hs-CRP in regression models. In a recent study from the United States National Health and Nutrition Examination Survey, CMV seropositivity was associated with hypertension in women; however, the association was not significant after adjusting for age.<sup>7</sup> In the Cardiovascular Risk in Young Finns Study, when considering traditional atherosclerotic risk factors, high CMV antibody titers were determinants for systolic and diastolic blood pressure elevation and brachial artery flow-mediated dilation in young men.<sup>8</sup> Regarding these conflicting human studies, we note a need for a complex approach that includes a package of new atherosclerotic risk factors to elucidate CMV's role in hypertension.

Our study revealed a significant association between IgG seropositivity for *H. pylori* and hypertension. This association was retained even after adjustment for conventional risk factors

for coronary artery disease. Our findings are consistent with 1 study showing a significant relationship between *H. pylori* infection and hypertension.<sup>13</sup> A significant decrease in blood pressure values, in particular in diastolic blood pressure values, was observed after eradication of *H. pylori* in patients affected by hypertension.<sup>32</sup> However, the results of a community-based, cross-sectional study indicated that *H. pylori* infection might not be a clinically significant associated factor for hypertension.<sup>33</sup>

The mechanism underlying the association of *H. pylori* infection and hypertension remains to be elucidated. A positive relationship between high salt intake and *H. pylori* infection was found in a multinational study.<sup>34</sup>

In a Swedish study of a defined population, subjects with combined positive serology for *H. pylori* and *C. pneumoniae* were characterized by lower social class and higher age, BMI, and fasting levels of insulin than the group without any positive serology.<sup>35</sup> The synergy of these 2 infections in the development of hypertension has not been investigated so far. However, seropositivity for these 2 organisms predicted increased risk of coronary artery disease, myocardial infarction, and higher CRP levels.<sup>36</sup>

Interestingly, we found that combined seropositivity for *H. pylori* and *C. pneumoniae*, compared with double

seronegativity, was independently associated with hypertension. Statistically, the contribution of this coinfection to hypertension was more pronounced than *H. pylori* IgG seropositivity alone. The *H. pylori* infection in combination with CMV or HSV-1 did not show a significant association with hypertension in this study. Hence, these findings may suggest a unique synergy of this coinfection in the development of hypertension, rather than the concept of pathogen burden in essential hypertension.

Shumacher *et al.*<sup>37</sup> demonstrated that coinfection with *H. pylori* and *C. pneumoniae* in patients with coronary artery disease was associated with elevated levels of soluble intercellular cell adhesion molecule-1 (sICAM-1) when compared with double seronegativity, which supports our findings. They also observed elevated levels of soluble vascular cell adhesion molecule 1 in this coinfection compared with those who were only *C. pneumoniae* immunoglobulin A positive, indicative of aggravation of the endothelial inflammatory response.<sup>37</sup> Additionally, Liuba *et al.*<sup>38</sup> showed that coinfection of apolipoprotein E-knockout mice with *H. pylori* and *C. pneumoniae* was associated with enhanced expression of VCAM-1 at the branching sites and impaired endothelial vasomotor function of thoracic aortas.

Our study has several limitations. Because our data are cross-sectional, limited inferences can be made regarding causality in combined *H. pylori* and *C. pneumoniae* infection in the development of high blood pressure. We conducted our study in a large, random population and used seropositivity as a marker for infections. Our study has the advantage of clinical applicability, but the assessment of infection status based on serology without further clinical or laboratory characterization is subject to diagnostic inaccuracies, especially if seropositivity is common because of widespread distribution of the microorganism in question. In our study, the presence of seropositivity to other confounding viral or bacterial pathogens cannot be ruled out. Furthermore, the measurement of additional atherosclerotic biological markers and cytokines merits consideration to elucidate the complexity that may underlie the interplay among infectious agents, inflammation, molecular mimicry, and structural changes in the arteries.

In conclusion, our study failed to show any significant association between burden of common viral and bacterial infectious pathogens that had been previously associated with human coronary atherosclerotic changes as well as carotid atherosclerosis and hypertension in a large, representative sample of Iranians. Although further studies using diverse populations are necessary to confirm our findings, our study refutes the hypothesis that the risk of essential hypertension is associated with aggregated pathogen load.<sup>15</sup> However, coinfection with *H. pylori* and *C. pneumoniae* appears to be associated with essential hypertension independently of conventional cardiovascular risk factors and hs-CRP levels.

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## DISCLOSURE

The authors declared no conflict of interest.

## REFERENCES

1. Rupprecht HJ, Blankenberg S, Bickel C, Rippin G, Hafner G, Prellwitz W, Schlumberger W, Meyer J. Impact of viral and bacterial infectious burden on long-term prognosis in patients with coronary artery disease. *Circulation* 2001; 104:25–31.
2. Vercellotti GM. Overview of infections and cardiovascular diseases. *J Allergy Clin Immunol* 2001; 108:S117–S120.
3. Kol A, Libby P. The mechanisms by which infectious agents may contribute to atherosclerosis and its clinical manifestations. *Trends Cardiovasc Med* 1998; 8:191–199.
4. Zhu J, Quyyumi AA, Norman JE, Csako G, Waclawiw MA, Shearer GM, Epstein SE. Effects of total pathogen burden on coronary artery disease risk and C-reactive protein levels. *Am J Cardiol* 2000; 85:140–146.
5. Elkind MS, Ramakrishnan P, Moon YP, Boden-Albala B, Liu KM, Spitalnik SL, Rundek T, Sacco RL, Paik MC. Infectious burden and risk of stroke: the northern Manhattan study. *Arch Neurol* 2010; 67:33–38.
6. Elkind MS. Infectious burden: a new risk factor and treatment target for atherosclerosis. *Infect Disord Drug Targets* 2010; 10:84–90.
7. Li C, Samaranayake NR, Ong KL, Wong HK, Cheung BM. Is human cytomegalovirus infection associated with hypertension? *The United States National Health and Nutrition Examination Survey 1999–2002*. *PLoS One* 2012; 7:e39760.
8. Haara A, Kahonen M, Lehtimäki T, Aittoniemi J, Jylhä J, Hutri-Kahonen N, Taittonen L, Laitinen T, Juonala M, Viikari J, Raitakari OT, Hurme M. Relation of high cytomegalovirus antibody titres to blood pressure and brachial artery flow-mediated dilation in young men: the Cardiovascular Risk in Young Finns Study. *Clin Exp Immunol* 2012; 167:309–316.
9. Pitiriga VCh, Kotsis VT, Alexandrou ME, Petrocheilou-Paschou VD, Kokolakis N, Zakopoulou RN, Zakopoulos NA. Increased prevalence of *Chlamydia pneumoniae* but not Epstein-Barr antibodies in essential hypertensives. *J Hum Hypertens* 2003; 17:21–27.
10. Blanc P, Corsi AM, Gabbuti A, Peduzzi C, Meacci F, Olivieri F, Lauretani F, Franceschi M, Ferrucci L. *Chlamydia pneumoniae* seropositivity and cardiovascular risk factors: the INCHIANTI Study. *J Am Geriatr Soc* 2004; 52:1626–1631.
11. Cook PJ, Lip GY, Davies P, Beevers DG, Wise R, Honeybourne D. *Chlamydia pneumoniae* antibodies in severe essential hypertension. *Hypertension* 1998; 31:589–594.
12. Sun Y, Pei W, Wu Y, Jing Z, Zhang J, Wang G. Herpes simplex virus type 2 infection is a risk factor for hypertension. *Hypertens Res* 2004; 27:541–544.
13. Lip GH, Wise R, Beevers G. Association of *Helicobacter pylori* infection with coronary heart disease. Study shows association between *H pylori* infection and hypertension. *BMJ* 1996; 312:250–251.
14. Tabib S, Vahdat K, Jafary S, Fiiiladvand M, Nabipour I, Sanjideh Z. Association between *H. pylori* infection & hypertension in the residents of the Northern Persian Gulf. *ISMJ* 2008; 10:159–165.
15. Liu L, Liu Y, Tong W, Ye H, Zhang X, Cao W, Zhang Y. Pathogen burden in essential hypertension. *Circ J* 2007; 71:1761–1764.
16. Nabipour I, Amiri M, Imami SR, Jahfari SM, Shafeiae E, Nosrati A, Iranpour D, Soltanian AR. The metabolic syndrome and nonfatal ischemic heart disease; a population-based study. *Int J Cardiol* 2007; 118:48–53.
17. Guidelines Subcommittee. 1999 World Health Organization–International Society of Hypertension guidelines for the management of hypertension. *J Hypertens* 1999; 17:151–183.
18. Shapiro DS, Loiacono LA. Mean arterial pressure: therapeutic goals and pharmacologic support. *Crit Care Clin* 2010; 26:285–293.

19. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 1997; 20:1183–1197.
20. Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001; 285:2486–2497.
21. Vahdat K, Azizi F, Zandi K, Assadi M, Nabipour I. Chronic inflammation is correlated with percentage of body fat independent of the burden of infection. *Inflammation* 2012; 35:1322–1329.
22. Rosenfeld ME, Campbell LA. Pathogens and atherosclerosis. Update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis. *Thromb Haemost* 2011; 106:858–867.
23. Elkind MS, Ramakrishnan P, Moon YP, Boden-Albala B, Liu KM, Spitalnik SL, Rundek T, Sacco RL, Paik MC. Infectious burden and risk of stroke: the northern Manhattan study. *Arch Neurol* 2010; 67:33–38.
24. Pauleto P, Rattazzi M. Inflammation and hypertension: the search for a link. *Nephrol Dial Transplant* 2006; 21:850–853.
25. Coles KA, Plant AJ, Riley TV, Smith DW, McQuillan BM, Thompson PL. Lack of association between seropositivity to *Chlamydia pneumoniae* and carotid atherosclerosis. *Am J Cardiol* 1999; 84:825–828.
26. Koh WP, Taylor MB, Hughes K, Chew SK, Fong CW, Phoon MC, Kang KL, Chow VT. Seroprevalence of IgG antibodies against *Chlamydia pneumoniae* in Chinese, Malays and Asian Indians in Singapore. *Int J Epidemiol* 2002; 31:1001–1007.
27. Nishimura M, Ushiyama M, Nanbu A, Mashida C, Kawagoe K, Yoshimura M. Inverse association of *Chlamydia pneumoniae* infection with high blood pressure in Japanese adults. *Am J Hypertens* 2001; 14:20–26.
28. Ibrahim AI, Obeid MT, Jouma MJ, Moasis GA, Al-Richane WL, Kindermann I, Boehm M, Roemer K, Mueller-Lantzsch N, Gartner BC. Detection of herpes simplex virus, cytomegalovirus and Epstein-Barr virus DNA in atherosclerotic plaques and in unaffected bypass grafts. *J Clin Virol* 2005; 32:29–32.
29. Siscovick DS, Schwartz SM, Corey L, Grayston JT, Ashley R, Wang SP, Psaty BM, Tracy RP, Kuller LH, Kronmal RA. *Chlamydia pneumoniae*, herpes simplex virus type 1, and cytomegalovirus and incident myocardial infarction and coronary heart disease death in older adults: the Cardiovascular Health Study. *Circulation* 2000; 102:2335–2340.
30. Kristensen BO, Andersen PL, Vestergaard BF, Andersen HM. Herpesvirus antibodies and vascular complications in essential hypertension. *Acta Med Scand* 1982; 212:375–377.
31. Cheng J, Ke Q, Jin Z, Wang H, Kocher O, Morgan JP, Zhang J, Crumpacker CS. Cytomegalovirus infection causes an increase of arterial blood pressure. *PLoS Pathog* 2009; 5:e1000427.
32. Migneco A, Ojetti V, Specchia L, Franceschi F, Candelli M, Mettimano M, Montebelli R, Savi L, Gasbarrini G. Eradication of *Helicobacter pylori* infection improves blood pressure values in patients affected by hypertension. *Helicobacter* 2003; 8:585–589.
33. Harvey R, Lane A, Murray L, Harvey I, Nair P, Donovan J. Effect of *Helicobacter pylori* infection on blood pressure: a community based cross sectional study. *BMJ* 2001; 323:264–265.
34. Beevers DG, Lip GY, Blann AD. Salt intake and *Helicobacter pylori* infection. *J Hypertens* 2004; 22:1475–1477.
35. Ekesbo R, Nilsson PM, Lindholm LH, Persson K, Wadström T. Combined seropositivity for *H. pylori* and *C. pneumoniae* is associated with age, obesity and social factors. *J Cardiovasc Risk* 2000; 7:191–195.
36. Anderson JL, Carlquist JF, Muhlestein JB, Horne BD, Elmer SP. Evaluation of C-reactive protein, an inflammatory marker, and infectious serology as risk factors for coronary artery disease and myocardial infarction. *J Am Coll Cardiol* 1998; 32:35–41.
37. Schumacher A, Seljeflot I, Lerkerod AB, Sommervoll L, Otterstad JE, Arnesen H. Does infection with *Chlamydia pneumoniae* and/or *Helicobacter pylori* increase the expression of endothelial cell adhesion molecules in humans? *Clin Microbiol Infect* 2002; 8:654–661.
38. Liuba P, Pesonen E, Paakkari I, Batra S, Andersen L, Forslid A, Yla-Herttuala S, Persson K, Wadstrom T, Wang X, Laurini R. Co-infection with *Chlamydia pneumoniae* and *Helicobacter pylori* results in vascular endothelial dysfunction and enhanced VCAM-1 expression in apoE-knockout mice. *J Vasc Res* 2003; 40:115–122.